

recombinant hypocretin protein can be present in or on a mammalian cell line or in crude extracts of a mammalian cell line.

[0054] In one embodiment, a hypocretin protein is substantially free of other neuropeptides, so that the purity of a hypocretin reagent, and thus freedom from pharmacologically distinct proteins, facilitates use in the screening methods. The recombinant production methods are ideally suited to produce significantly improved purity in this regard, although biochemical purification methods from natural sources are also included. In this regard, a hypocretin protein is substantially free from other neuropeptides if there are insufficient other neuropeptides such that pharmacological cross-reactivity is not detected in conventional screening assays for ligand binding or biological activity. Alternatively, recombinant hypocretin fusion proteins can be produced by joining nucleotides encoding additional amino acid residue sequence in proper reading frame at the 3' end of the hypocretin sequence. The fusion protein thus produced exhibits properties of the added amino acid sequence in addition to the properties of hypocretin. For example, the additional amino acid sequence may serve to help identify and purify the recombinantly produced hypocretin fusion protein. One preferred hypocretin fusion protein is hypocretin-poly(His).

[0055] Preferably, a hypocretin protein of this invention is present in a composition in an isolated form, i.e., comprising at least about 0.1 percent by weight of the total composition, preferably at least 1%, and more preferably at least about 90%. Particularly preferred is a substantially pure preparation of hypocretin, that is at least 90% by weight, and more preferably at least 99% by weight. Biochemical methods useful for the enrichment and preparation of an isolated hypocretin based on the chemical properties of a polypeptide are well known, and can be routinely used for the production of proteins which are enriched by greater than 99% by weight.

[0056] An isolated or recombinant hypocretin protein of this invention can be used for a variety of purposes, as described further herein. A hypocretin protein can be used as an immunogen to produce antibodies immunoreactive with hypocretin. Hypocretin proteins can be used in *in vitro* ligand binding assays for identifying ligand binding specificities, and agonists or antagonists thereto, to characterize candidate pharmaceutical compounds useful for modulating hypocretin function, and as therapeutic agents for effecting hypocretin functions. Other uses will be readily apparent to one skilled in the art.

[0057] Furthermore, the invention includes analogs of a hypocretin protein of this invention. An analog is a man-made variant which exhibits the qualities of a hypocretin of this invention in terms of immunological reactivity, ligand binding capacity or the like functional properties of a hypocretin protein of this invention. An analog can therefore be a cleavage product of hypocretin, can be a polypeptide corresponding to a portion of hypocretin, can be hypocretin polypeptide in which a membrane anchor has been removed, and can be a variant hypocretin sequence in which some amino acid residues have been altered, to name a few alternatives.

[0058] Insofar as the present disclosure identifies hypocretin from different mammalian species, the present inven-

tion is not to be limited to a hypocretin protein derived from one or a few mammalian species. Thus, the invention includes a mammalian hypocretin protein, which can be derived, by recombinant DNA or biochemical purification from natural sources, from any of a variety of species including man, mouse, rabbit, rat, dog, cat, sheep, cow, and the like mammalian species, without limitation. Human and agriculturally relevant animal species are particularly preferred.

[0059] Exemplary hypocretin species identified herein are rat and mouse hypocretin.

[0060] The amino acid residue sequence of rat preprohypocretin is shown in SEQ ID NO 1, and corresponding nucleotide (cDNA) of rat preprohypocretin is shown in SEQ ID NO 3.

[0061] The amino acid residue sequence of mouse preprohypocretin is shown in SEQ ID NO 2, and corresponding nucleotide (cDNA) of mouse preprohypocretin is shown in SEQ ID NO 4.

[0062] A hypocretin protein of this invention can be prepared by a variety of means, although expression in a mammalian cell using a recombinant DNA expression vector is preferred. Exemplary production methods for a recombinant hypocretin are described in the Examples.

[0063] The invention also provides a method for the production of isolated hypocretin proteins, either as intact hypocretin protein, as fusion proteins or as smaller polypeptide fragments of hypocretin. The production method generally involves inducing cells to express a hypocretin protein of this invention, recovering the hypocretin from the resulting cells, and purifying the hypocretin so recovered by biochemical fractionation methods, using a specific antibody of this invention, or other chemical procedures.

[0064] The inducing step can comprise inserting a recombinant DNA vector encoding a hypocretin protein, or fragment thereof, of this invention, which recombinant DNA is capable of expressing a hypocretin, into a suitable host cell, and expressing the vector's hypocretin gene.

[0065] As used herein, the phrase "hypocretin polypeptide" refers to a polypeptide having an amino acid residue sequence that comprises an amino acid residue sequence that corresponds, and preferably is identical, to a portion of a hypocretin of this invention.

[0066] A hypocretin polypeptide of this invention is characterized by its ability to immunologically mimic an epitope (antigenic determinant) expressed by a hypocretin of this invention. Such a polypeptide is useful herein as a component in an inoculum for producing antibodies that immunoreact with native hypocretin and as an antigen in immunologic methods. Representative and preferred hypocretin polypeptides for use as an immunogen in an inoculum are shown herein.

[0067] As used herein, the phrase "immunologically mimic" in its various grammatical forms refers to the ability of a hypocretin polypeptide of this invention to immunoreact with an antibody of the present invention that recognizes a conserved native epitope of a hypocretin as defined herein.

[0068] It should be understood that a subject polypeptide need not be identical to the amino acid residue sequence of a hypocretin receptor, so long as it includes the required sequence.